Original Research Article

Chemical comparison in stem and leaves of*Cupressussempervirens*(cypress) ofCubanorigin

Comment [A1]: Botanical name incomplete and local name should be used later; not in title

ABSTRACT

Comment [A2]: Make it simple and in lucid language for common man understanding

Cupressussempervirens, known as ¨Ciprés¨, no other species studied is comparable with this almost exclusive possibility of being able to concentrate and bind secondary metabolites, mineral salts; millennial culture, mystical and enigmatic traditions of immortality. There are specimens with more than 1000 years of longevity, almost virginal exuberance of their natural landscapes, spectacular images in an attempt to rediscover a past that does not lose its ancestry; not offered by other plants. It is of widespread popular use worldwide and multiple applications in different economic spheres; It is native to the Mediterranean areas. In Cuba it is a

rarity; This is the reason why the present investigation begins with the objective of comparing the chemical characterization of the Cuban ¨Ciprés¨ in stem and leaves. The Cuban ¨Ciprés belongs to the Cupressaceae families, of interest for research at the Institute of Pharmacy and Food, University of Havana.The Maceration Method is applied for the extraction of its chemical constituents qualitatively determined by Phytochemical Sieving; expressing with greater incidence essential oils, fats, alkaloids, triterpenoids and steroids, resins, reducing sugars, saponins, flavonoids, phenolic compounds, mucilage, and bitter principles. The High-Resolution Liquid Chromatography suggests that possibly the compounds that presented retention time in the stem of 15.2 and 15.9 minutes and those with retention time in leaves of 15.2 and 16.0 minutes, respectively, are two compounds that represent the same type of metabolites, in each case, produced by the plant in each part of the plant. Compounds present in leaves that do not appear on stems are shown and vice versa.

Keywords:Cupressussempervirens, chemical composition, Cypress, Mediterranean

1. INTRODUCTION

Medicinal plants have several beneficial conditions for humanity, one of them is their medicinal contribution due to the presence of phytochemicals and antioxidants, characterized by these bioactive compounds as the main source of nutraceuticals [1]. Furthermore, they are considered a source of vitamins and minerals [2]; therefore, they can be used in the food industry for the elaboration of functional foods [3] contributing to the development of new products, with a positive economic and social impact [4].

C. sempervirens L is a tree of European origin (figure 1) [5] [6]; with medicinal properties and potential pharmacological interest, it is known in the world as cypress and cypress, its cultivation is ornamental, it maintains a height between 10 and 20 m in height, it has smooth bark, grayish color, it has a long and narrow crown, fusiform, with upright branches and small leaves, empirically the fruits and bark have been used to control stomach problems, it has also been used in the treatment of parasites, as an anti-abortion and as an insect repellent, in Cuba they are grown in addition to the Mediterranean cypress, other species among which *C. arizonica* Greene, *C. macrocarpa*Martweg, *C. lusitanica* Mill., *C. funebris*Lindl stand out. *C. torulosaa* Don, *C. Benthami*Endl.and*C. glabra*Sudwort [7].

Figure 1.*C. sempervirens*of Cuban origin.

2. MATERIAL AND METHODS

The experimentation work was carried out in the Synthesis Laboratories of the Pharmacy Department at the Institute of Pharmacy and Food (IFAL), University of Havana. The plant material of *C. sempervirens* (Cypress) used in this research is made up of fresh stems and leaves.The material was collected at the Novartis Laboratories Commercial House in Vedado, Plaza de la Revolution Municipality in the province of Havana, Cuba, with herbarium number HAC43083.

2.1 Washing and disinfection of the drug

The washing was carried out with abundant demineralized water, immersed in 1% sodium hypochlorite for five minutes, and then washed for ten minutes [8].

2.2 Macroscopic evaluation of the drug

The macroscopic evaluation of the plant was performed by determining the quality control parameters [8]. For the characterization of the leaves, the following parameters were determined: size, color, odor, shape, condition, and surface characteristics.

Comment [A3]: ????????

2.3 Preparation of samples and extracts

Fresh samples were micronized at a particle size between 0.8 to 2 mm in a FUMAR brand knife mill (Germany). The extracts of the leaves and stems of the species *C. sempervirens* (Cypress) were prepared with samples of 50 g by the method of maceration with 95% ethanol (Class A) for 7 to 14 days at room temperature and shaking in a shaker for qualitative characterization in HPLC equipment. In all cases, reagent grade solvents and the solutions corresponding to each test were used.

Comment [A4]: Sample preparation method??

2.4 Phytochemical screening

Phytochemical screening was carried out through identification reactions through a color change, flavor identifier, the formation of precipitates [8], from the evaluation of essential oils and fats, saponins, polysaccharides, resins, alkaloids, lactones and coumarins, cardiotonic glycosides, phenols, triterpenes, and steroids, reducing compounds, amino acids and amines, quinones, flavonoids, and anthocyanidins.

2.5 High-Resolution Liquid Chromatography (HPLC) Chromatographic Separation

Comment [A5]: Full form of HPLC is wrong

Samples of stem and leaves of the species *C. sempervirens* (Cypress) were analyzed by HPLC using a KNAUER Chromatograph (Germany) with a C-18 column and dilution of the sample by eight times. The HPLC chromatographic separation, with a 280 nm UV detector, consisting of an Azura P6.1L low-pressure quaternary gradient pump, an Azura UVD 2.1L UV-Vis detector, an Azura CT 2.1 Thermostat, and a manual injector. The identification and integration of peaks were performed using a computer compatible with the HPLC Control Program for the acquisition and manipulation of chromatographic data, ClarityChrom version 6.1.0.130 (KNAUER, Germany). Chromatographic separation was performed using a Eurospher C-18 reverse phase column, 60 A, 5 µm (d.i), 250 x 4 mm, of German manufacture. HPLC chromatographic analysis was carried out using water as eluent A and acetonitrile and a gradient from 15 to 85% B as eluent B, for forty minutes at 1mLmin-1

followed by maintenance of the gradient, increasing A by 50%, for ten sustained minutes, reverting to 0% B for five minutes, and rebalancing for five minutes at a temperature of 25 0C. In the analyzes carried out, we used extracts made with fresh cypress plants at concentrations of 95% (v / v) in ethanol.

3. RESULTS AND DISCUSSION

3.1Phytochemical Screening Results

The "screening" techniques allow to qualitatively detect the presence of certain groups of compounds, which rely on microchemistry to show these groups of constituents through the formation of precipitates, colorations, flavor, among others. The screening was carried out on two parts of the fresh plant of the same species *C. sempervirens*(Cypress), one with the leaves (Table 1) of intense green color and the other with the stem (Table 2) to observe if the results of both samples from the same plant were similar in terms of qualitative chemical composition or differed.

The results of the two phytochemical screenings of *C. sempervirens* (Cypress) show the presence of bioactive compounds that make the plant of interest to the pharmaceutical industry, these results, as well as the tests carried out are shown in Tables 1 and 2. experimentally found that the most significant results in both screenings were those of Dragendorff, Libermann-Burchard, Ferric Chloride, and Shinoda. Both phytochemical screenings arise the presence of fatty compounds, essential oils, alkaloids, triterpenes and/or steroids, reducing sugars, saponins, phenolic compounds, flavonoids, anthocyanins, and mucilages. Therefore, both screenings show the presence of some metabolites common in both parts of the plant and others not, except forsaponins that are reported in the leaves and not in the stem.

Table 1.Results of phytochemical screening in leaves of *C. sempervirens*

Tests	*Metabolites*	*Results*

		Etheral	*Alcoholic*	*Aqueous*
Sudan	Fatty compounds	+++		
Dragendorff	Alkaloids	+++	-	+++
Baljet	Lactonic grouping	-	-	
Libermanm.B	Triterpenes / steroids	+++	+++	
Catequins	Catechins		-	
Resins	Resins		+++	
Fehling	Reducing sugars		+++	+++
Foam	Saponins		+++	+++
Chloride Fe	Phenolic compounds		+++	-
Ninhydrin	Free amino acids		-	
Borntrager	Benzoquinquinones		-	
Shinoda	Flavonoids		+++	+++
Kedde	Cardiotonic glycosides		-	
Anthocyanidin	Anthocyanins		-	
Mucilages	Mucilages			+++
Beginnings A	Beginnings A			+++
Essentialoils	Essential oils	+++		

Tests	*Metabolites*	*Results*

Table 2.Results of the phytochemical screening in stems of *C. sempervirens*

		Etheral	*Alcoholic*	*Aqueous*
Sudan	Fatty compounds	+++		
Dragendorff	Alkaloids	+++	-	+++
Baljet	Lactonic grouping	-	-	
Libermanm.B	Triterpenes / steroids	+++	+++	
Catequins	Catechins		-	
Resins	Resins		-	
Fehling	Reducing sugars		+++	+++
Foam	Saponins		-	-
Chloride Fe	Phenolic compounds		+++	-
Ninhydrin	Free amino acids		-	
Borntrager	Benzoquinquinones		-	
Shinoda	Flavonoids		+++	-
Kedde	Cardiotonic glycosides		-	
Anthocyanidin	Anthocyanins		-	
Mucilages	Mucilages			+++
Beginnings A	Beginnings A			+++
Essentialoils	Essential oils	+++		

3.2 Analysis of the Chromatogram of the Ethanolic Extract of the Stem of *C. sempervirens* (Cypress)

Through the analysis of the two extracts by high-performance liquid chromatography (HPLC), it was observed that in the case of the stem extract, a simple chromatogram was obtained with the isocratic water / ACN elution system with the C-18 column and 8-fold dilution of the sample, detecting 44 components, with the presence of two very significant chromatographic peaks between 15,167 and 15,883 minutes, obtaining the highest

percentage of relative area for the peaks that eluted between 9,500 and 15,883 minutes (Figure 2). The major compounds, or those that present the greatest interest in the chromatographic profile of the hydroalcoholic extracts of the stems, are those that elute at 10.4; 11.0; 11.3; 11.7; 12.4; 13.3; 15.2; 15.9; 16.4; 17.9; 20.5; 25.6; 27.6; 29.9 and 36.6 minutes, respectively. The two major compounds showed a retention time of 15.2 and 15.9 minutes, respectively.

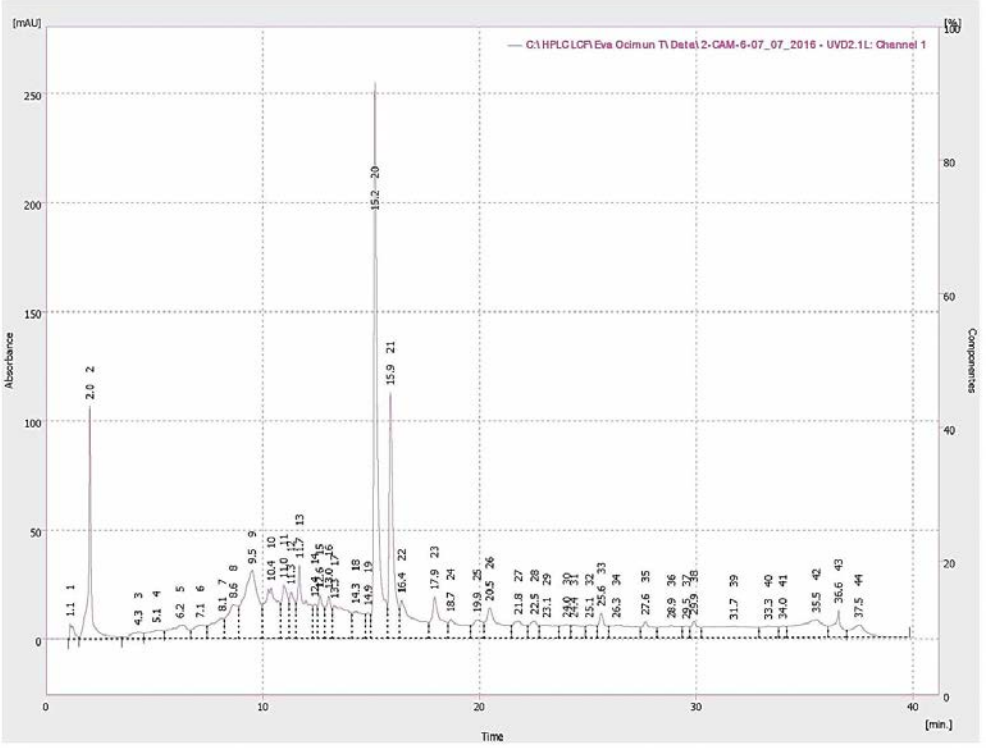

Figure *2.*Chromatograms of the ethanolic extract of the stem of *C. sempervirensfrom Cuba*

3.3 Analysis of the Chromatogram of the Ethanolic Extract of the Leaves of *C.sempervirens*(Cypress)

In the case of the leaves, a simple appearance was also shown, although visualizing the increase in complexity in them, nine significant chromatographic peaks were shown, obtaining the highest percentage of the relative area (40.2%) for the peak that eluted at 11.8 minutes. The compounds of greatest interest in the chromatographic profile of the leaves of the plant are those that eluted at 10.1; 11.1; 11.8; 12.7; 15.2; 16.0; 18.0; 20.4 and 36.6 minutes, respectively. In this case, the majority compounds are those that eluted at 11.8; 15.2 and 16.0 minutes, respectively (figure 3).

It is suggested that possibly the compounds that presented a stem retention time of 15.2 and 15.9 minutes and those with a leaf retention time of 15.2 and 16.0 minutes, respectively, are two compounds that represent the same type of metabolite, in each case, produced by the plant in each part of the plant that has been analyzed in this study. It also shows compounds present in leaves that do not appear on stems and vice versa.

It is inferred, therefore, that the ethanolic extract of the leaves is the one that contains the greatest amount of compounds (47 compounds) according to the results achieved under the test conditions used, compared to the stem that contains (44 compounds).

When comparing the two extracts of the two parts of the plant material, leaves and stem, differences were observed in the High-Resolution Liquid Chromatography, with the stem extract being the one with the most complex chromatograms.

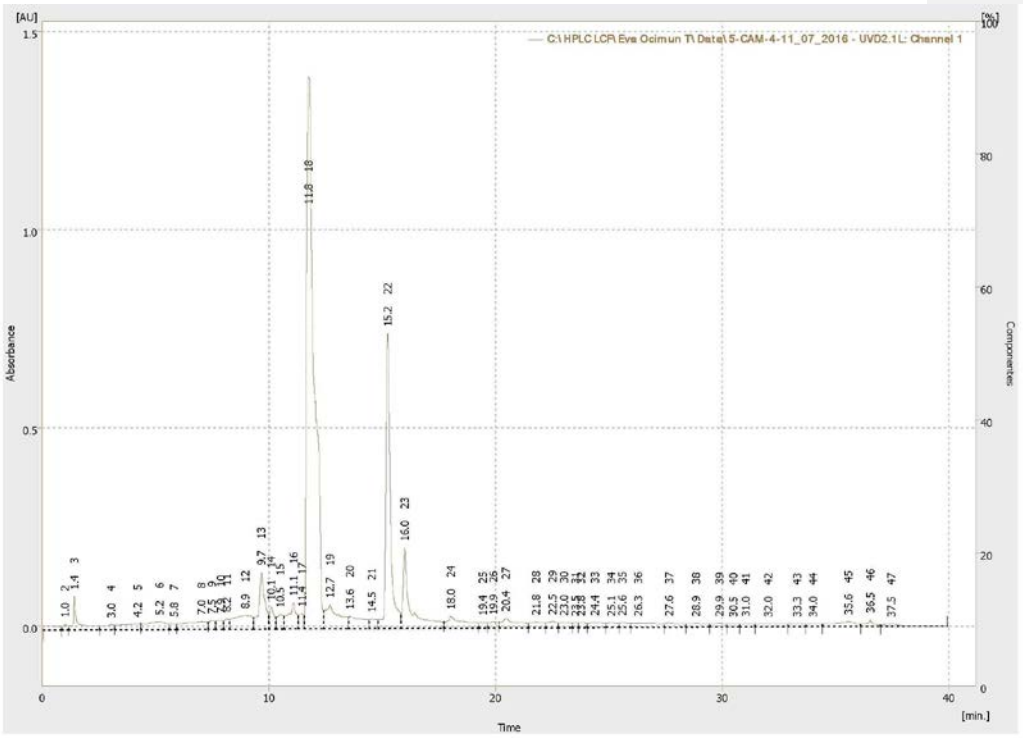

Figure 3. Chromatograms of the ethanolic extract of the leaves of C. sempervirens from Cuba

3.4. Discussion of the Pharmacological Importance of some of the metabolites determined experimentally in C. sempervirens (Cypress) of Cuban origin

Alkaloids

The alkaloids expressed in leaves and stem of Cuban C. sempervirens have pharmacological properties depending on the molecular configuration, such as: for sympatholytics and antispasmodics, local anesthetics and sources of drug addiction, they act as anticholinergics, that is, in gastric motility disorders and Muscle spasm, especially in some ulcer patients and as central depressants of motor activity, with an anthelmintic effect,

a very positive result for the present evaluation. It has vasoconstrictive effects due to its action on the sympathetic ganglia and to promote the release of vasopressin and adrenaline. In high doses, they can become toxic) [9, 10, 11]

Simple phenols

In the results in leaves and stem of C. sempervirens of the test with ferric chloride, the presence of an intense green coloration was observed, for which the possible presence of condensed tannins can be inferred.

The presence of phenols is because these compounds are closely related to the activity of repellency by plants to insect populations and since leaves are the most common target, it is logical that they are used in chemical defense. Due to their antioxidant properties, these compounds have shown beneficial effects on human health as anti-inflammatory, anti-sclerotic, and antiviral [12].

Flavonoids

The flavonoid result was positive in the hydroalcoholic extract of leaves and stem of C. sempervirens showing a phase separation with a brown color, which is indicative of flavonoids of flavones and flavonols. These metabolites have been shown to exhibit a wide range or spectrum of pharmacological and biochemical actions such as antimicrobial, antithrombotic, antimutagenic, and anti-carcinogenic activities [13, 14]. The activity of flavonoids as antioxidants depends on the redox properties of their hydroxyphenolic groups and the structural relationship between the different parts of the chemical structure. Flavonoids, in particular, exhibit a wide range of biological effects, including antiviral, anti-inflammatory, antiallergic, antioxidant, and vasodilator activity [11, 15].

The C. sempervirenscypress, is another species that has great variability in the chemical composition, reported with indifference but that in summary coincide with the global results

described in the present work, which for the first time differentiates two parts (leaves-stem) of the same variety for the present analysis; both parts (stem-leaves) of the evaluated plant material have chemically active properties for the pharmaceutical use of the plant species Cupressussempervirens, ¨ciprés¨ of Cuban origin [13].

4. CONCLUSION

C. sempervirens of Cuban origin is an antiseptic, antirheumatic, antispasmodic, astringent, anti-sudorific, diuretic, neuronal restorer, taking into account the presence of important and dissimilar chemical constituents of great pharmaceutical value. It could be used in baths, showers, massages, cosmetics, inhalations, compresses, evaporators, among others, given by the varied constituent chemical composition; both the leaves and the stem.Besides, these results serve as a guide and orientation on which secondary metabolites may be responsible for the biological effects suggested for this species and the extraction method that should be applied to them where they are expressed.

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